Insulin Resistance Is Attenuated in Women with Polycystic Ovary Syndrome with the Pro12Ala Polymorphism in the PPARγ Gene

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Polycystic ovary syndrome (PCOS) is common in women of reproductive age and is associated with a high risk for development of type 2 diabetes. Insulin resistance, a key component in the pathogenesis of PCOS and glucose intolerance, is ameliorated by the thiazolidinediones, synthetic ligands for the PPARγ. In the present study we have examined the relationship of the Pro12Ala polymorphism in the PPARγ gene (PPARG) to clinical and hormonal features of PCOS.

Two hundred and eighteen women with PCOS had a 75-g oral glucose tolerance test, and blood was obtained for measurement of serum androgen levels. Sixty percent of the subjects were Caucasian, 26% were African-American, 6% were Hispanic, 6% were South Asian, and 2% were Middle-Eastern. Compared with Caucasians, the African-American group had a higher prevalence of diabetes (19% vs. 5%, respectively), were more obese (body mass index, 40.9 ± 1.8 vs. 36.3 ± 0.8 kg/m²; P < 0.05), and were more insulin resistant.

Twenty-eight of 218 subjects had the Ala allele, all in the heterozygous state. The frequency of the Ala allele varied among the groups: 0.01 in African-Americans, 0.08 in Caucasians, and 0.15 in Hispanics. Nondiabetic Caucasians with an Ala allele (Pro/Ala group) were more insulin sensitive than those in the Pro/Pro group, as evidenced by a lower homeostasis model assessment index (5.18 ± 1.33 vs. 6.54 ± 0.54; P < 0.05) and lower levels of insulin at both the fasting (132 ± 27 vs. 165 ± 12 pmol/liter; P = 0.03) and 2 h (688 ± 103 vs. 10190 ± 99 pmol/liter; P = 0.04) time points during the oral glucose tolerance test. We conclude that Pro12Ala in PPARG is a modifier of insulin resistance in Caucasian women with PCOS.

Subjects and Methods

Subjects

Subjects were recruited from the endocrinology clinics of the University of Chicago. All were at least 2 yr postmenarche and less than 40 yr of age. A diagnosis of PCOS required 1) the presence of oligo/amenorrhea; 2) hyperandrogenemia, defined by a supranormal plasma free T level (≥34.7 pmol/liter); 3) hyperandrogenism, as evidenced by infertility, hirsutism, acne, or androgenetic alopecia; and 4) exclusion of nonclassic 21-hydroxylase deficiency congenital adrenal hyperplasia, Cushing’s syndrome, hypothyroidism, or significant elevations in serum PRL (2). In addition to meeting these diagnostic criteria for PCOS, often referred to as the NIH consensus criteria (20), each subject had hormonal evidence of ovarian androgen overproduction documented by an abnormal 17-hydroxyprogesterone response to GnRH agonist administration or a supranormal plasma free T level after administration of dexamethasone (2). For at least 2 months before the study, subjects had not taken steroid preparations (including oral contraceptives) or medications known to alter insulin secretion and/or action. These studies were approved by the institutional review board of the University of Chicago, and written informed consent was obtained from each participant.

Oral glucose tolerance testing

All individuals, with the exception of those known to be diabetic, had an oral glucose tolerance test (OGTT). After an overnight 12-h fast, blood samples were obtained at −15 and 0 min. A glycohemoglobin level was also obtained at 0 min. Dextrose (75 g) was then administered orally, and blood samples were obtained at 30, 60, 90, and 120 min for measurement of glucose and insulin concentrations. Glucose tolerance status was based upon the plasma glucose concentration at 2 h using the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of the American Diabetes Association (21). Specifically, a diagnosis of normal glucose tolerance, impaired glucose tolerance, or
diabetes was assigned if the glucose level at 2 h was less than 7.8 mmol/liter, between 7.8–11.1 mmol/liter, or 11.1 mmol/liter or more, respectively.

**Hormonal measures**

Serum was obtained from each subject for measurement of total T, free T, SHBG, and dehydroepiandrosterone sulfate (DHAS).

**Molecular genetic studies**

Exon 2 of PPARγ2 was amplified using PCR and the primers G2F (5'-CIGATGCTTGCATCTGCG-3') and G2R (5'-CGGAAGCAACATCAAGAGC-3'). The 295-bp PCR product was digested overnight with Hgal, which cleaves the Ala allele to generate DNA fragments 178 and 117 bp in size. The DNA fragments were separated on 3% agarose gel.

**Assay methods**

Plasma glucose was measured immediately using a glucose analyzer (model 2300 STAT, YSI, Inc., Yellow Springs, OH). The coefficient of variation of this method is less than 2%. Glycosylated hemoglobin was measured by boronate affinity chromatography with an intraassay coefficient of variation of 4% (Bio-Rad Laboratories, Inc., Hercules, CA). Serum insulin was assayed by a double antibody technique (22) with a lower limit of sensitivity of 20 pmol/liter and an average intraassay coefficient of variation averaged 6%.

Plasma T was measured using a kit from Diagnostic Products (Los Angeles, CA). The free fraction of plasma T and the concentration of SHBG were measured by a competitive protein binding assay as previously described (22). The lower limit of sensitivity of the assay is 0.02 pmol/ml, and the intraassay coefficient of variation averaged 6%.

SHBG was measured by a competitive protein binding assay as previously described (22). Between-group comparisons were made using Hgal, which cleaves the Ala allele to generate DNA fragments 178 and 117 bp in size. The DNA fragments were separated on 3% agarose gel.

**Statistical analysis**

All statistical analyses were performed using StatView software (SAS Institute, Inc., Cary, NC). Between-group comparisons were made using ANOVA or analysis of covariance with logarithmic transformation of data not normally distributed and post-hoc correction for multiple comparisons. P < 0.05 was considered significant. All data are presented as the mean ± se.

**Results**

**Clinical characteristics of the study population (Table 1)**

Two hundred and eighteen PCOS subjects were examined: 130 (60%) Caucasian of European ancestry, 57 (26%) African-Americans, 13 (6%) Hispanics, 13 (6%) South Indians, and 5 (2%) of Middle Eastern ancestry. Twenty-two (10%) of the subjects were diabetic, consistent with the increased prevalence of diabetes in PCOS (4, 5). African-Americans accounted for 11 (50%) of the diabetes cases; the remaining half was comprised of 6 (27%) Caucasians, 3 (14%) Hispanics, 1 (5%) South Indian, and 1 (5%) Middle Eastern.

The groups were similar in age, but differed in BMI. African-Americans (BMI, 40.9 ± 1.8 kg/m²) and Hispanics (BMI, 39.2 ± 1.5 kg/m²) were significantly more obese than the three other groups. In comparison to Caucasians, African-American women had significantly higher levels of insulin at both the fasting (225 ± 22 pmol/liter) and 2 h measurements (1641 ± 223 pmol/liter) during the OGTT as well as higher glucose levels at 2 h (8.5 ± 0.4 mmol/liter). Finally, both total (2.69 ± 0.13 mmol/liter) and free (83.9 ± 4.9 pmol/liter) T levels were significantly higher in African-Americans compared with both Caucasian and Hispanic subjects. When comparisons were performed using BMI as a covariate, the differences in fasting insulin and HOMA index between Caucasian and African-American subjects were no longer significant. However, significance was retained for the differences in 2 h insulin, 2 h glucose, and total and free T between African-Americans and Caucasians after adjusting

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>African-American</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hispanic</td>
<td>South Indian</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>109</td>
<td>56</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>103</td>
<td>45</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Diabetic</td>
<td>6</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pro/Ala</td>
<td>21</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ala allele frequency (±se)</td>
<td>0.08 ± 0.017</td>
<td>0.01 ± 0.009</td>
<td>0.15 ± 0.07</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30.3 ± 0.6</td>
<td>28.4 ± 1.0</td>
<td>28.8 ± 2.2</td>
<td>25.8 ± 1.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.3 ± 0.8</td>
<td>40.9 ± 1.8a</td>
<td>39.2 ± 1.5</td>
<td>30.3 ± 1.7b</td>
</tr>
<tr>
<td>Glycohemoglobin (%)</td>
<td>5.8 ± 2.0</td>
<td>5.7 ± 0.1</td>
<td>5.5 ± 0.3</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>Fasting glucose (mmol/liter)</td>
<td>5.2 ± 0.1</td>
<td>5.3 ± 0.2</td>
<td>5.7 ± 0.3c</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>2 h glucose (mmol/liter)</td>
<td>7.6 ± 0.5</td>
<td>8.5 ± 0.4d</td>
<td>8.2 ± 0.8</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>Fasting insulin (pmol/liter)</td>
<td>161 ± 11</td>
<td>225 ± 22e</td>
<td>241 ± 47</td>
<td>172 ± 21</td>
</tr>
<tr>
<td>2 h insulin (pmol/liter)</td>
<td>1031 ± 83</td>
<td>1641 ± 223f</td>
<td>1287 ± 257</td>
<td>1570 ± 340</td>
</tr>
<tr>
<td>HOMA</td>
<td>6.5 ± 0.5</td>
<td>9.3 ± 1.1e</td>
<td>9.9 ± 1.7f</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>Total T (nmol/liter)</td>
<td>2.68 ± 0.13</td>
<td>3.43 ± 0.25b</td>
<td>2.25 ± 0.27</td>
<td>2.83 ± 0.25</td>
</tr>
<tr>
<td>Free T (pmol/liter)</td>
<td>83.9 ± 4.9</td>
<td>105.7 ± 9.0b</td>
<td>67.6 ± 6.9</td>
<td>91.2 ± 8.7</td>
</tr>
<tr>
<td>SHBG (nm)</td>
<td>16.8 ± 1.9</td>
<td>14.9 ± 1.1</td>
<td>14.9 ± 3.2</td>
<td>10.8 ± 1.8</td>
</tr>
<tr>
<td>DHAS (nmol/liter)</td>
<td>5.1 ± 0.3</td>
<td>4.4 ± 0.4</td>
<td>4.4 ± 0.6</td>
<td>6.4 ± 1.3</td>
</tr>
</tbody>
</table>

Data are the mean ± sem. Normal ranges: glycohemoglobin, <7.2%; fasting glucose, ≤6.1 mmol/liter; 2 h glucose, <7.8 mmol/liter; total T, 0.66–2.43 nmol/liter; free T, 10.4–34.7 pmol/liter; SHBG, 12–63 nm; dehydroepiandrosterone, 1.9–9.9 μmol/liter.

a P < 0.05 vs. Caucasian, South Indian, and Middle Eastern.
b P < 0.05 vs. Caucasian and Hispanic.
c P < 0.05 vs. Caucasian.
for BMI. The differences in free T levels could not be accounted for by differences in levels of SHBG.

**PPARG and PCOS**

The Pro<sup>12</sup>Ala polymorphism was typed in all 218 PCOS subjects (Table 1). Twenty-eight had the Ala allele, all in the heterozygous state. The frequency of the Ala allele varied among the groups and ranged from 0.01 in African-Americans to 0.15 in Hispanics (Table 1). These values are similar to those determined in previous studies (13).

The effect of the Ala allele on clinical and hormonal measures was next analyzed in the nondiabetic Caucasian group (Table 2). There were too few subjects with the Ala allele to allow similar comparisons in the other groups. Diabetic subjects were excluded to eliminate the confounding effect of the diabetic state on the measures analyzed. There was no apparent effect of the Pro<sup>12</sup>Ala genotype on BMI. However, subjects with the Ala allele were more insulin sensitive, as evidenced by a significantly lower HOMA index (5.18 ± 1.33 vs. 6.54 ± 0.54; P < 0.05) as well as significantly lower levels of insulin at both the fasting (132 ± 27 vs. 165 ± 12 pmol/liter; P = 0.03) and 2 h (688 ± 103 vs. 10190 ± 99 pmol/liter; P = 0.04) points during the OGTT (Table 2). This difference remained significant after adjusting for the effect of BMI.

Finally, both total and free T levels were lower in those women with the Ala allele, but the difference from those with the Pro/Pro genotype was not statistically significant.

**Table 2.** Clinical and hormonal characteristics of nondiabetic Caucasian PCOS subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of subjects</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>Glycohemoglobin (%)</th>
<th>Fasting glucose (mmol/liter)</th>
<th>2 h glucose (mmol/liter)</th>
<th>Fasting insulin (pmol/liter)</th>
<th>2 h insulin (pmol/liter)</th>
<th>HOMA</th>
<th>Total T (mmol/liter)</th>
<th>Free T (pmol/liter)</th>
<th>SHBG (nM)</th>
<th>DHA5 (µmol/liter)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro/Pro</td>
<td>103</td>
<td>30.4 ± 0.8</td>
<td>36.1 ± 0.8</td>
<td>5.9 ± 0.2</td>
<td>5.2 ± 0.1</td>
<td>7.6 ± 0.3</td>
<td>165 ± 12</td>
<td>1090 ± 99</td>
<td>6.54</td>
<td>2.84 ± 0.15</td>
<td>89.4 ± 5.9</td>
<td>15.9 ± 1.7</td>
<td>5.33 ± 0.35</td>
<td>0.49</td>
</tr>
<tr>
<td>Pro/Ala</td>
<td>21</td>
<td>29.2 ± 1.3</td>
<td>33.9 ± 1.9</td>
<td>5.5 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>7.1 ± 0.3</td>
<td>132 ± 27</td>
<td>688 ± 103</td>
<td>5.18</td>
<td>2.31 ± 0.22</td>
<td>68.9 ± 6.9</td>
<td>14.1 ± 1.5</td>
<td>4.08 ± 0.54</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Normal ranges: glycohemoglobin, <7.2%; fasting glucose, ≤6.1 mmol/liter; 2 h glucose, ≤7.8 mmol/liter; total T, 0.66–2.43 nmol/liter; free T, 10.4–34.7 pmol/liter; SHBG, 12–63 nM; DHA5, 1.9–9.9 µmol/liter.

**Discussion**

The etiology of PCOS is complex and incompletely understood (3, 7). However, it is generally recognized that insulin resistance is a key component in the pathogenesis of the disorder (7), and several reports have documented the association of PCOS with genes influencing insulin action (24, 25). The Pro<sup>12</sup>Ala polymorphism in PPARG has been implicated in the pathogenesis of other insulin-resistant conditions, including type 2 diabetes and obesity (13).

We found that Caucasian women with PCOS who had the Ala allele at Pro<sup>12</sup>Ala, although still substantially insulin resistant, were less insulin resistant than those with two Pro alleles. This difference could not be accounted for by a difference between groups in BMI, as both Pro/Ala and Pro/Pro subjects were similar in their degree of obesity (BMI, 33.9 ± 1.9 vs. 36.1 ± 0.8 kg/m²; P = 0.31). Further, when measures of insulin resistance were compared by analysis of covariance with BMI as a covariate, the results were similar.

These results are consistent with other studies in non-PCOS subjects, indicating that the presence of an Ala allele is associated with increased insulin sensitivity (11, 12). Our results suggest that the Pro<sup>12</sup>Ala polymorphism in PPARG is among the factors affecting insulin resistance in Caucasian women with PCOS. This polymorphism does not appear to contribute to the variation in insulin resistance among African-American women with PCOS because the frequency of the Pro allele approaches 1.0 in this population. Further studies are needed to define its role in other groups.

The Pro allele at Pro<sup>12</sup>Ala has been associated with a modest 1.25-fold increased risk of diabetes, but because the risk allele occurs with such high frequency, its modest effect translates into a large population-attributable risk, estimated to influence as much as 25% of type 2 diabetes in the general population (13). Studies of a larger group of PCOS women will be necessary to assess the effects of the Pro<sup>12</sup>Ala polymorphism on the risk of type 2 diabetes in this extremely insulin-resistant group of subjects.

**Acknowledgments**

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**References**


